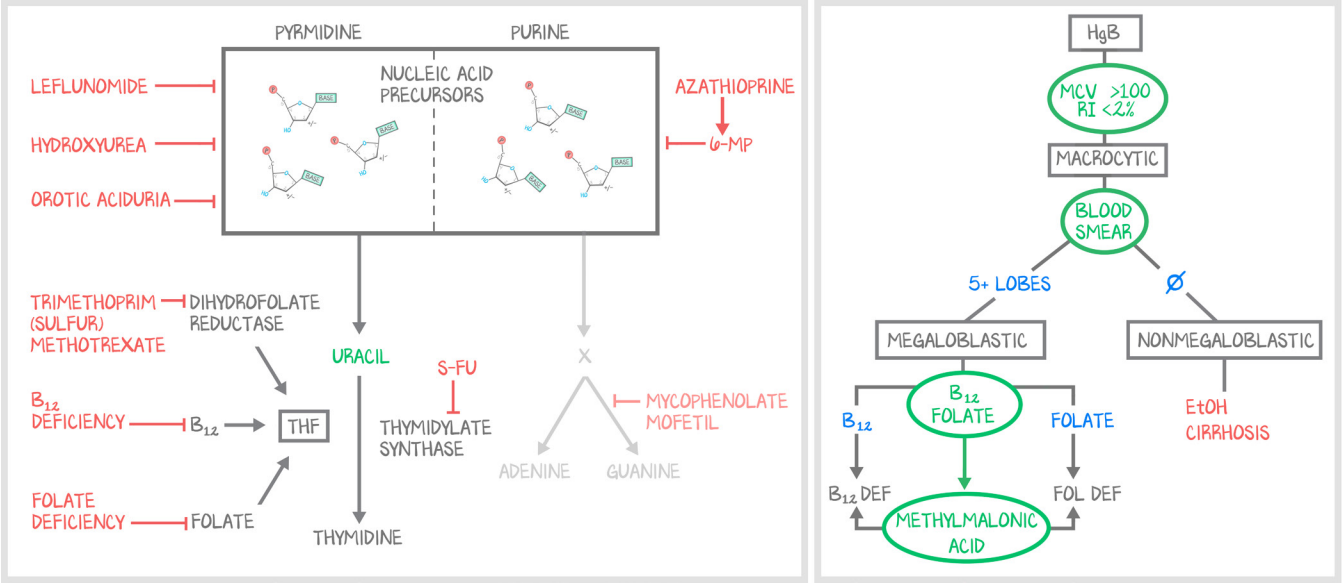
Macrocytic Anemia

Introduction

Macrocytic anemia is an anemia (low hemoglobin) where the red cells are large (MCV > 100). Except for cirrhosis and chronic alcoholism, all the causes of macrocytic anemia are also megaloblastic. Megaloblastic anemia derives from impaired nucleic acid synthesis. There are other conditions in which the red blood cells will be reported as high (as in when reticulocytes are released in response to hemolytic anemia), but the reticulocytes are supposed to be larger than mature differentiated erythrocytes. In other words, a large erythrocyte is either a product of cirrhosis or alcohol, or it's a product of impaired DNA synthesis. We're going to spend our time on impaired DNA synthesis.

We know the patient is anemic because they have a low hemoglobin on the complete blood count. The mean corpuscular volume is elevated (**MCV > 100**) and the reticulocyte index confirms a production problem (**reticulocyte index < 2%**). Macrocytic anemia is the diagnosis. On the **blood smear** if there is evidence of hypersegmented neutrophils, any neutrophil with five or more lobes, the macrocytic anemia is said to be **megaloblastic**. All megaloblastic anemias come down to **impaired nucleic acid synthesis**. Any inhibition of any nucleic acid synthesis will result in a megaloblastic anemia. The vast majority of this lesson is in regard specifically to the synthesis of the nucleic acid thymidine, made from uracil. The disorders of thymidine synthesis can be divided into two broad categories—insufficient **folate** and insufficient **substrate**. All of the hoopla found on that pathway comes down to dUMP being converted to dTMP: deoxy-uracil-mono-phosphate into deoxy-thymidine-mono-phosphate—turning uracil into thymidine. That step is carried out by **thymidylate synthase**, adding a methyl group to uracil, making uracil thymidine.



(a) (b)

Figure 6.1: Macrocytic Anemia
(a) The simplified versions of purine and pyrimidine synthesis and the medications that impact them. Any impaired nucleic acid synthesis will result in megaloblastic anemia. (b) Diagnostic algorithm for macrocytic anemias.

The **substrate** for that enzyme is uracil. Anything that decreases the amount of uracil will provoke a megaloblastic anemia. **Orotic aciduria** (a disease you will not see after Step 1) is an enzymatic deficiency that leads to underproduction of uracil. Many **drugs** inhibit a reaction somewhere along the

complicated pyrimidine synthesis pathway. Examples include leflunomide and hydroxyurea. One drug, **5-fluorouracil**, inhibits thymidylate synthase directly.

In order to function, thymidylate synthase requires a cofactor in addition to the substrate uracil—tetrahydro**folate** (THF). The generation of THF from dietary folate requires B_{12}. Therefore, the **folate diseases** are all a product of either **folate deficiency** (which naturally would obviously result in tetrahydrofolate deficiency) and **B_{12} deficiency** (which also causes a tetrahydrofolate deficiency, though in a less obvious way).

We'll explore this in intense detail in the next section, but here's a high-level view. To turn uracil into thymidine, thymidine synthase adds a single methyl group to uracil. THF grabs a methyl group from another thing we aren't naming on purpose, and then gives it to uracil. The dietary folate, the folate our bodies can be absorbed through our GI tract, enters as a form that must first be converted to THF. Dietary folate is turned into the good THF by giving up a methyl group to B_{12}. You did read that correctly. Dietary folate gives a methyl group to B_{12} to become THF so it can get a different methyl group from something else to give a methyl group to uracil. More on this in a bit.

DNA is, of course, more than just thymidine. Megaloblastic anemia can be seen in any condition in which any nucleotide synthesis is inhibited. For example, as you learned in Immunology #16: *Immunosuppression*, there are medications that inhibit inflammation by preventing proliferation of lymphocytes. They work by targeting the purine synthesis pathway: 6-mercaptopurine, azathioprine, and mycophenolate mofetil, which also would cause a megaloblastic anemia. These are summarized in Figure 6.1.

The Nitty Gritty

Refer to Figure 6.2 as you read through this text. The words are long and complicated. But really, the only thing that happens is that methyl groups get shuffled around.

5-Methyl-THF is how our gut absorbs folic acid, how we get folate into us. That 5-methyl-THF carries the methyl that B_{12} uses to methylate homocysteine, making methionine. The dietary 5-methyl-THF becomes the THF needed for thymidylate synthase by donating its methyl to B_{12}. B_{12} takes the methyl from 5-methyl-THF and gives it to homocysteine. 5-Methyl-THF becomes THF, and homocysteine becomes methyl-homocysteine, formerly known as methionine.

The dietary 5-methyl-THF gave its methyl group to B_{12}, making regular THF. THF does not now have a methyl group to give. Yet we know thymidylate synthase adds a methyl group to uracil to make thymidine, and it uses a methylated THF to do it. So the 5-methyl-THF became regular THF, and now (in an enzymatic reaction purposefully left unnamed), regular THF gets methylated to 5,10-methylene-THF. This enzymatic reaction does not involve B_{12} in any way, nor the methyl that B_{12} took from 5-methyl-THF. It instead takes a methyl group from a serine and gives it to THF, making 5,10-methylene-THF.

That 5,10-methylene-THF can take one of two paths. In one path, it can become 5-methyl-THF again and thus give its methyl to B_{12}. In the other path, it can it could give its methyl group directly to uracil, forming thymidine. If this is the path 5,10-methylene-THF takes, the methyl group is donated to uracil, and 5,10-methylene-THF becomes DHF (dihydro-folate). An enzyme called **DHF reductase** turns the DHF back into the THF that can be methylated by a serine, to make 5,10-methylene-THF once again.

In a **folate deficiency** there is no new dietary folate (5-methyl-THF) around to give its methyl to B_{12} and become THF. If there is no new source of THF, and since there are other uses of folate in the body, there won't be enough THF to keep up with nucleic acid synthesis. Uracil cannot be turned into thymidine because there is no 5,10-methylene-THF, and B_{12} doesn't have a source of methyl to give to

homocysteine. Nucleic acid synthesis slows, and the patient gets a megaloblastic anemia. Because there is no source of methyl to give to B_{12}, there is no way of giving homocysteine a methyl group to become methionine. Therefore, homocysteine levels accumulate.

In a **B_{12} deficiency**, there is nothing to take the 5-methyl-THF's methyl group, and folic acid dead-ends at 5-methyl-THF. The dietary folate cannot be turned into the useful-for-nucleic-acid-synthesis regular THF. So a B_{12} deficiency causes a relative folate deficiency. But not just any folate—a THF deficiency. And, if there is no B_{12}, even if there are massive amounts of 5-methyl-THF being absorbed from the diet, there is nothing to transfer a methyl to homocysteine. With a relative THF deficiency, nucleic acid synthesis slows, and the patient gets a megaloblastic anemia. Because there is no B_{12} to give homocysteine a methyl, homocysteine accumulates.

In both B_{12} and folic acid deficiencies, homocysteine levels rise. In both B_{12} and folic acid deficiencies, there is impaired nucleic acid synthesis and megaloblastic anemia. So how do you tell them apart? You can do that with the labs and with the clinical picture. The way to do that with the labs is with **methylmalonic acid (MMA)**; B_{12} deficiency has an elevated MMA; folate deficiency does not. The enzymatic reaction involved has nothing to do with the system discussed, it is a separate enzymatic reaction and is something you must commit to memory. The way you would separate them clinically is with **spinal cord findings**; B_{12} deficiency results in loss of sensation and motor; folate deficiency does not. That is how the board will test you, providing a megaloblastic anemia and asking you to separate the two. But we also want you understanding something profound when you practice—B_{12} deficiency takes a decade, and the causes are so obvious systemically that you should not be seeing a patient starting with their B_{12} deficiency; folate deficiency takes weeks and happens all the time.

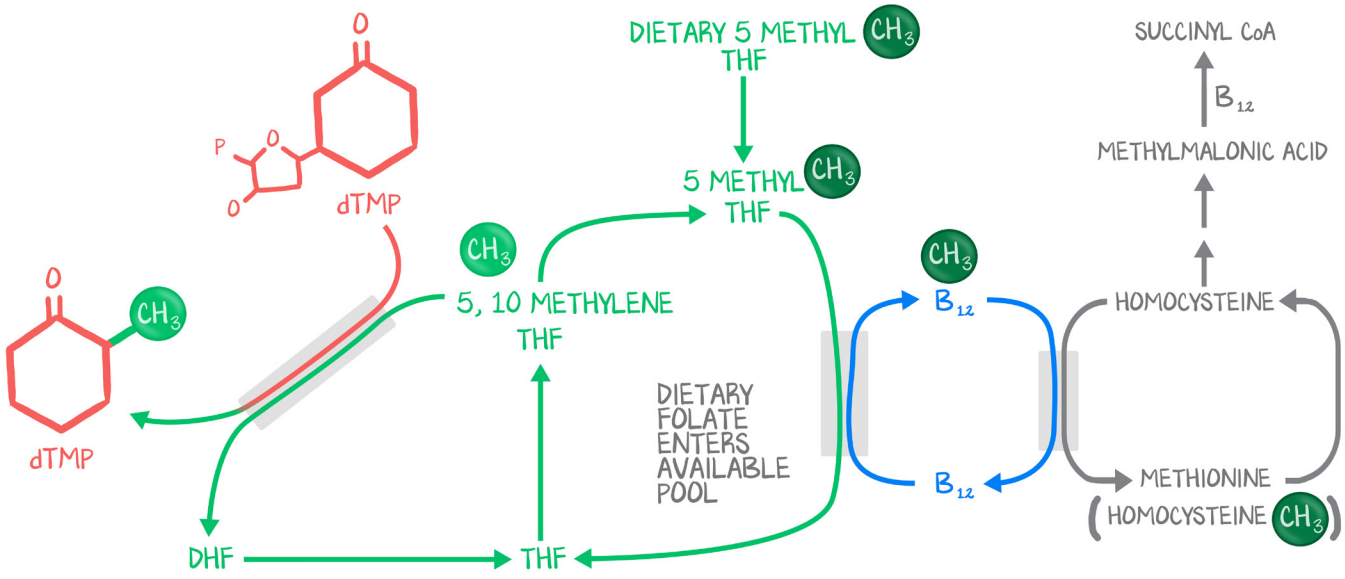


Figure 6.2: Pyrimidine Synthesis and Vitamin Deficiency
Pyrimidine synthesis simplified. Megaloblastic anemia is about DNA synthesis. The holdup is getting enough uracil (technically in the deoxyribose monophosphate form) and enough tetrahydrofolate, THF (technically in its 5,10-methylene-THF form), so that thymidylate synthase can take a methyl from THF and give it to uracil, making thymidine. 5-methyl-THF- gives its methyl to B_{12} becoming THF. B_{12}-methyl then gives that methyl to homocysteine, making methionine (which is just homocysteine-methyl). The THF goes off to get 5,10-methylene'd and will give its methyl to uracil to form thymidine. In addition to methylating homocysteine, B_{12} is used to facilitate myelin synthesis and the conversion of methylmalonic acid to succinyl-CoA. In folate deficiency and B_{12} deficiency, homocysteine accumulates. Only in B_{12} deficiency does methylmalonic acid accumulate and is myelin synthesis impaired.

The Effects of Impaired DNA on Bone Marrow

Erythrocyte precursors in the bone marrow divide many times before terminally differentiating into erythrocytes. Erythrocytes have no nucleus or mitochondria. Erythrocyte precursors do. Cell division (mitosis) requires that the DNA be replicated in its entirety before progressing out of synthesis phase into G_2. DNA polymerase normally has an abundance of all DNA nucleic acids in the nucleus, so is not rate limited. If there is a paucity of thymidine, DNA polymerase must wait for a thymidine to be available in order to pair it with adenine. This means that the phase of synthesis takes longer than usual. Longer replication time means that the erythrocyte precursor will undergo fewer divisions until terminal differentiation. The time to terminal differentiation is independent of the number of divisions. Every time an erythrocyte precursor divides, it gets a little smaller. If there is a prolonged replication time, aka **fewer divisions** (say, for example, because of a paucity of nucleic acids needed for S phase of mitosis), there will be **larger erythrocytes**. That's how we get the macrocytic anemia part of megaloblastic macrocytic anemia.

Neutrophil precursors in the bone marrow develop their cytoplasm and their nucleus through separate mechanisms but at the same time. Immature neutrophils, called blasts, are the final stage before release from the bone marrow. The development of the nucleus requires nucleic acids. The development of the cytoplasm does not. Since the cytoplasm matures faster than the nucleus, and neutrophils are multilobed phagocytes, **five or more lobes in a neutrophil nucleus** indicates that there is impaired DNA synthesis. The neutrophil still works; it's just that the nucleus doesn't look how it is supposed to look when there isn't a thymidine deficiency. That's how we get the megaloblastic part of megaloblastic macrocytic anemia.

Getting the Uncommon Ones out of the Way

Orotic aciduria is an extremely uncommon **autosomal recessive** deficiency of UMP synthase (that's the non-deoxy version). It is upstream of the dUMP used by thymidylate synthase, but we want you seeing it as "just another substrate disease." The presentation is hard to miss—impaired DNA synthesis at birth doesn't make for great development in a neonate. The neonate will either be diagnosed by **prenatal genetic screen**, or present with the combination of macrocytic megaloblastic anemia and **failure to thrive** with significant developmental delay. Diagnosis is confirmed by finding **orotic acid** in the **urine** (thus the name of the diagnosis). Treatment is by giving them UMP, downstream of the defective enzyme.

Medications can cause megaloblastic anemia. **6-Mercaptopurine**, **mycophenolate mofetil**, and **hydroxyurea** all somehow impair synthesis of nucleic acids. **5-Fluorouracil** targets thymidylate synthase directly. **Trimethoprim-sulfamethoxazole** and **methotrexate** inhibit dihydrofolate reductase, resulting in a relative folate deficiency by reducing the generation of THF from DHF. Regardless of where these drugs influence nucleic acid synthesis, there will be a reduced number of some nucleic acid, and therefore impaired DNA synthesis.

Folate Deficiency

Folic acid is an essential vitamin (B_9). Bacteria are able to synthesize it; humans cannot. It is abundant in the diet, especially in **leafy greens**. The liver serves as the reservoir for folate, but has only **3–6 weeks of storage** available. It is folate deficiency, whether true (low folate levels) or relative (B_{12} deficiency, medications, above), that results ultimately in impaired thymidine synthesis. Because there is so little reserve in the liver, folate runs out whenever there is increased demand or decreased input. **Increased demand** comes in the way of increased red blood cell turnover (hemolytic anemia) or as blood volume increases in pregnancy. Chronic hemolytic anemias need regular folate supplementation to ensure that the normocytic anemia from hemolysis does not induce a secondary macrocytic anemia from folate deficiency. **Decreased input** is seen in patients who do not consume normal meals. Since many foods are fortified with folate, and most vegetables have folate, it is really hard not to get folate in the United States. Those who manage to be folate deficient from their diet alone will appear on board examinations

as **chronic alcoholics** (who get all their calories from alcohol) or as a **recent widower** (who, without his wife, cannot cook for himself, so eats a "tea and toast" diet). In actual patients, those who are at risk of folate deficiency have an **impaired jejunum**, where folate is absorbed. Conditions such as **celiac sprue** or jejunal resection from Crohn's disease can result in impaired intestinal absorption. **Pregnant women** and patients with hemoglobinopathies, who have increased demand for RBC production, also are at risk.

As discussed above in "The Nitty Gritty," the laboratory diagnosis of folate deficiency is a **megaloblastic anemia** (5+ lobes), a **low folic acid level**, and a **high homocysteine**. Did you hear anything about methylmalonic acid? No. Folate deficiency has nothing to do with methylmalonic acid. If you don't already know, you will be fully edumacated in the next section.

Treatment is to **replete folate**. Oral supplementation is usually sufficient.

B_{12} Deficiency

Vitamin B_{12} is only available to humans in the form of animal products. The human consumes the animal. In the animal that was just swallowed, B_{12} is attached to a protein in the animal that binds to B_{12}. In the stomach, **pepsin** degrades the binding protein. **Haptocorrin** (R-factor) is secreted in salivary tissue along with the meal, which then binds to the freed B_{12} in the stomach, forming B_{12}-R. In addition, **parietal cells** secrete **intrinsic factor (IF)**. In the duodenum, **pancreatic enzymes** degrade the haptocorrin, releasing the B_{12}. In the **jejunum**, intrinsic factor and B_{12} form a complex. In the **terminal ileum**, there are **intrinsic factor receptors** on the apical domain of enterocytes. Activation induces endocytosis. This happens with or without the B_{12} attached.

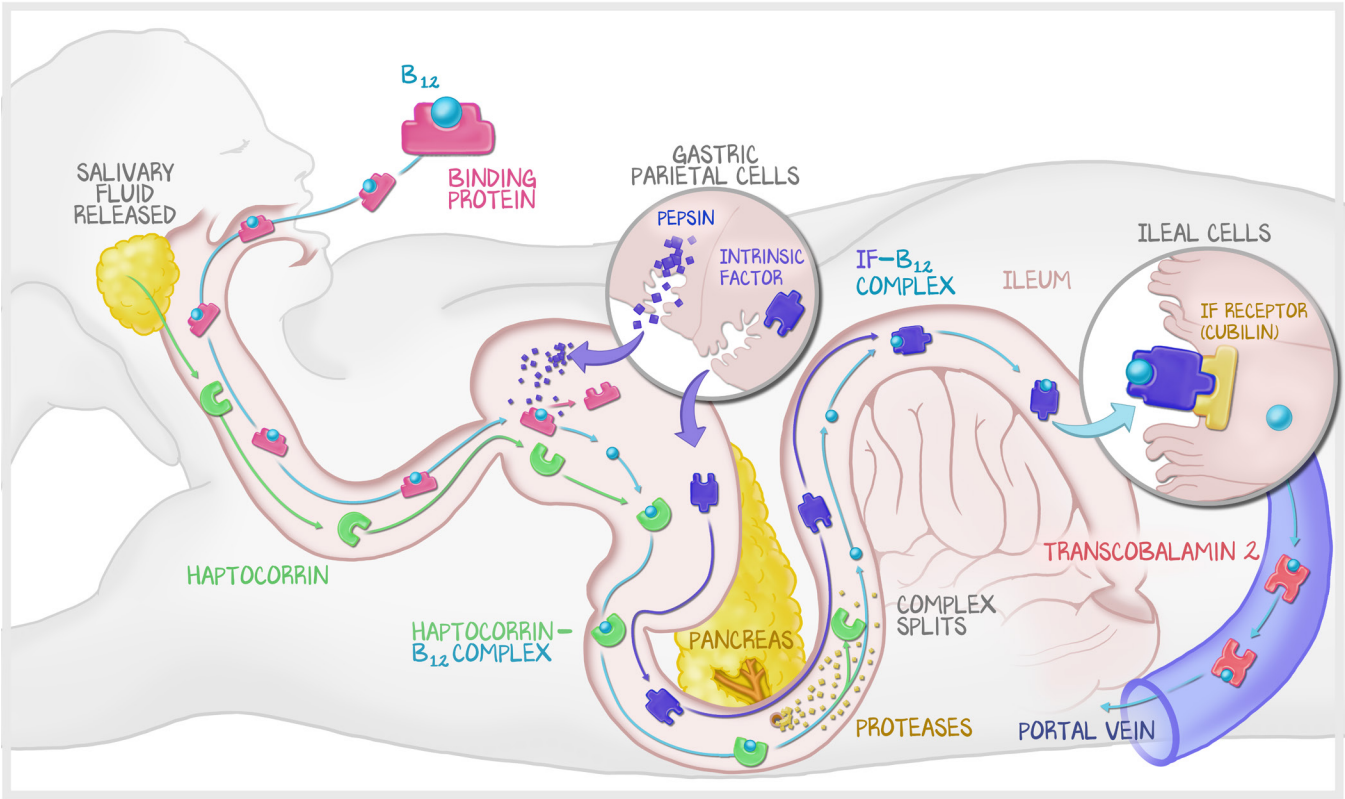


Figure 6.3: B_{12} Absorption
An illustration showing all the steps of B_{12} ingestion, digestion, and absorption. The key players are B_{12}, parietal cells in the gut secreting pepsin and acid, the pancreas with proteases, and enterocytes of the terminal ileum with their intrinsic factor receptors.

What all that means is there are A LOT of things that can go wrong in the absorption of B_{12}. All of those steps must happen, all of those tissues must be intact, in order to absorb the B_{12}. The good news is that, unlike folate, B_{12} has **a decade's worth of reserves** in the liver, and any diet other than absolute strict veganism will come with so much B_{12} that it is never a dietary issue. To say it clearly, the only way to have dietary B_{12} deficiency is to be vegan, to be uneducated in the need for B_{12} supplementation as a vegan, and to be a strict yet uneducated vegan for a very long time. No other human eating food has this issue. Therefore, it is highly unlikely that B_{12} deficiency comes from dietary deficiency. Instead, it comes from any number of anatomic or functional defects.

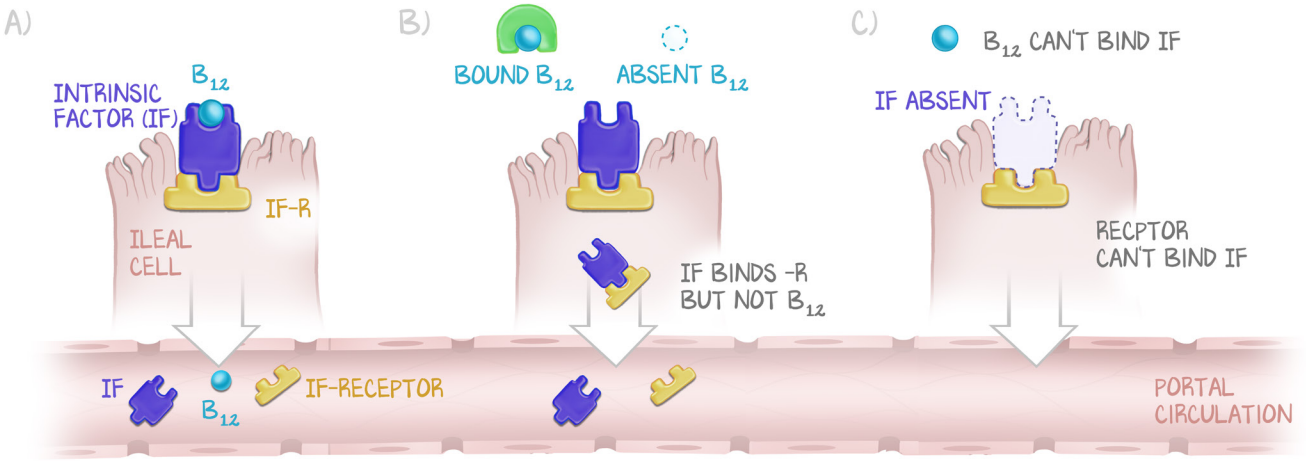


Figure 6.4: B_{12} Absorption and Pathogenesis
(a) What normally happens—IF/B_{12} binds the intrinsic factor receptor and both are absorbed. (b) Insufficient pancreatic enzymes result in the B_{12}-haptocorrin complex failing to be separated, so B_{12} cannot bind to IF. IF binds and is absorbed normally, but without B_{12}. (c) If no receptor is activated but B_{12} is ingested, B_{12} will not be absorbed.

Pernicious anemia was discussed in GI. Antibodies against parietal cells and antibodies against intrinsic factor are released into the stomach and bloodstream. This causes an atrophic gastritis, and, without intrinsic factor, vitamin B_{12} cannot be absorbed. **Pancreatic insufficiency** (to the extent of complete loss of exocrine pancreas function) can be responsible. In patients with chronic pancreatitis so severe as to cause B_{12} deficiency (such as cystic fibrosis patients), their pancreatic insufficiency is so severe that it would not be the B_{12} deficiency that draws attention to their pancreas. Finally, **Crohn's disease** can cause issues with B_{12} absorption, especially if inflammation or stricture required **resection of the terminal ileum**. Remember, a Crohn's flare cannot prevent absorption enough to drain the 10-year supply of B_{12} in the liver. Any insult must be chronic, prolonged, and therefore likely overt. In Asia and Africa, infection with Diphyllobothrium latum, a tapeworm, can steal the intrinsic factor and B_{12} along with other nutrients. That is not something you will see in the United States.

Should a patient develop B_{12} deficiency, they will of course suffer from the megaloblastic macrocytic anemia. That megaloblastic anemia is because of a relative folate deficiency. As discussed above, 5-methyl-THF, an unusable form except by B_{12}, accumulates, trapping folate in a dead-end molecular state. But B_{12} is used for more than just restoring THF and thus facilitating nucleic acid synthesis. **B_{12} deficiency causes loss of myelin**, which presents as **subacute combined degeneration of the cord**. There is first a demyelination of the dorsal column's medial lemniscus (vibration sense, proprioception) and lateral corticospinal (motor control, coordination) tracts. Ongoing deficiency results in permanent death of axons. **B_{12}-deficiency-induced neural symptoms are irreversible.** It is so obvious, the way we have taught megaloblastic anemia ("*it's either B_{12} deficiency or folate deficiency . . . or one of those other things that you told me don't matter*") that we have made it impossible for you ever to make this next mistake. **If you give folate to B_{12} deficiency, the anemia will get better but the irreversible neural symptoms will worsen.**

You should have the algorithm humming in the back of your brain. If MCV > 100, blood smear. If megaloblastic anemia, get folate and B_{12}. If not sure, get methylmalonic acid. What we also hope you take away is that, in practice, if the MCV > 100, and since B_{12} and folate levels cost pennies to run, you order a B_{12} and folate reflexively on every macrocytic patient you encounter.

What is this deal about methylmalonic acid? Completely separate from the discussion about THF, nucleic acids, and myelin, is an unrelated reaction in biochemistry. It had something to do with amino acid catabolism, right? The context is irrelevant. All you need to know is that the enzymatic reaction that takes **methylmalonic acid** and turns it into succinyl-CoA **requires B_{12}**, but not folate. That means in a B_{12} deficiency, the substrate accumulates, and there is an **elevated methylmalonic acid**. If ever you have labs that are equivocal (B_{12} and folate are low, but not obviously low), get the methylmalonic acid. If the methylmalonic acid is elevated, it is B_{12} deficiency. If the methylmalonic acid is not elevated, it is folate deficiency.

What about homocysteine? Since both folate and B_{12} are required for the homocysteine-to-methionine step, **homocysteine will be elevated in both folate deficiency and B_{12} deficiency**, so cannot be used to distinguish between the two.

Replete **B_{12}**, orally if they can, intramuscularly if they cannot.

Schilling Test

Why this is still a thing, we are not sure. However, this remains a subject of licensure examinations. The Schilling test is designed to take a person with B_{12} deficiency, and, without doing a history and physical and without having other labs to evaluate the patient, attempt to deduce the cause behind their B_{12} deficiency. Think how ludicrous that sounds. The person with cystic fibrosis has pancreatic insufficiency, the person with Crohn's who has their ileum removed has that cause in their surgical history, pernicious anemia is diagnosed with intrinsic factor antibodies, and the strict vegan has a dietary history. And yet, because the ingestion of B_{12} is so complicated, and the Schilling test evaluates every cause of B_{12} deficiency, it is still used to assess your knowledge of B_{12} absorption. You will never use this test. You may see it on your exam. The test begins by **saturating liver B_{12} receptors** with an intramuscular dose of B_{12}. Then, with each step, radiolabeled B_{12} is ingested and the urine assessed. Because there is no place for B_{12} to go (the IM B_{12}-saturated hepatic receptors), the radiolabeled B_{12} is urinated out as excess. If absorbed, it will end up in the urine. If not absorbed, it will not end up in the urine.

ADMINISTER	IF URINE POSITIVE	WHAT YOU SHOULD HAVE DONE
B_{12} orally	Oral absorption intact	Eaten more B_{12}
B_{12} orally and IF	Pernicious anemia	Parietal cell antibodies
B_{12} orally and Abx	Intestinal overgrowth	Wait . . . which antibiotic?
B_{12} orally and pancreatic enzymes	Pancreatic insufficiency	Recognized the crippling bouts of recurrent pancreatitis or the incredibly short stature, pulmonary infections, and obviously positive genetic screen of the CF patient

Table 6.1